A genetic linkage map for Arctic char (*Salvelinus alpinus*): evidence for higher recombination rates and segregation distortion in hybrid versus pure strain mapping parents

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Abstract: We constructed a genetic linkage map for Arctic char (*Salvelinus alpinus*) using two backcrosses between genetically divergent strains. Forty-six linkage groups (expected = 39–41) and 19 homeologous affinities (expected = 25) were identified using 184 microsatellites, 129 amplified fragment length polymorphisms (AFLPs), 13 type I gene markers, and one phenotypic marker, SEX. Twenty-six markers remain unlinked. Female map distance (9.92 Morgans) was substantially higher than male map distance (3.90 Morgans) based on the most complete parental information (i.e., the F_1 hybrids). Female recombination rates were often significantly higher than those of males across all pairwise comparisons within homologous chromosomal segments (average female to male ratios within families was 1.69:1). The female hybrid parent had significantly higher recombination rates than the pure strain female parent. Segregation distortion was detected in four linkage groups (4, 8, 13, 20) for both families. In family 3, only the largest fish were sampled for genotyping, suggesting that segregation distortion may represent regions possessing influences on growth. In family 2, almost all cases showing segregation distortion involved markers in the female hybrid parent.

Key words: salmonid fishes, polyploidy, homeology, genetic markers.

Résumé : Les auteurs ont produit une carte de liaison génétique pour l'omble chevalier (*Salvelinus alpinus*) à l'aide de deux rétrocroisements entre lignées génétiquement divergentes. Quarante-six groupes de liaison (l'attente était de 39–41) et 19 groupes homéologues (attente = 25) ont été identifiés à l'aide de 184 microsatellites, 129 AFLP, 13 marqueurs de gènes de type 1 et un marqueur phénotypique, SEX. Vingt-six marqueurs sont restés non-assignés. La distance totale sur la carte femelle (9,92 Morgans) était significativement plus grande que celle de la carte mâle (3,90 Morgans) en s'appuyant sur l'information génétique la plus complète sur les parents (c'est-à-dire les hybrides F_1). Les taux de recombinaison chez la femelle étaient souvent significativement plus élevés que chez le mâle pour toutes les comparaisons deux à deux au sein de segments chromosomiques homologues (en moyenne le ratio femelle : mâle était de 1,69 : 1). Le parent hybride femelle a montré significativement plus de recombinaison que la lignée femelle parentale pure. De la distorsion de la ségrégation a été détectée chez quatre groupes de liaison (4, 8, 13 et 20) chez les deux familles. Au sein de la famille 3, seuls les plus gros poissons ont été génotypés ce qui suggère que la distorsion pourrait refléter l'influence de certaines régions génomiques sur la croissance. Au sein de la famille 2, presque tous les cas de distorsion de la ségrégation impliquaient des marqueurs chez le parent hybride femelle.

Mots clés : poissons salmonidés, polyploïdie, homéologie, marqueurs génétiques.

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Introduction

Linkage map construction facilitates a number of important biological investigations including comparative mapping among species, searching for genes controlling phenotypic, life history, and physiological traits (e.g., quantitative trait loci), and the study of evolutionary processes. These include differences in genome structuring, recombination rates, and segregation patterns (i.e., segregation distortion) between the sexes, individuals within populations, and populations and strains of a species or between closely related species. The organization of genes into linkage groups may represent sets of wholly or partially interrelated "functional units" that may be under similar evolutionary selection pressures and may in fact be important determinants of speciation events and not merely consequences thereof (King 1993). The conservation of orthologous gene clusters ranging from fish (zebrafish, pufferfish, medaka) to humans (Ton et al. 2000; Nonaka et al. 2001; Santagati et al. 2001; Grutzner et al. 2002; Ringholm et al. 2002) suggests many syntenies of potential evolutionary significance.

Patterns of genetic recombination within species have the potential to influence the degree of conservation of allelic complements following meiosis (Burt 2000). Evidence is now starting to accumulate in humans that the genome may be structured in haplotype blocks that experience decreased rates of recombination interspersed with regions experiencing higher than average recombination (Gabriel et al. 2002). Such cases demonstrate that selection may operate on closely linked clusters of genes preserving distinct allelic assemblages within such clusters, at least within certain regions of the genome (e.g., major histocompatability complex; Jeffreys et al. 2001). The physical arrangement of gene order within a linkage group may, therefore, define chromosomal blocks that experience varying rates of crossing over. Dependent on the sex of the individual, the population of the individual, selection on allelic complements, and the dynamics of crossing over within the chromosomal region, varying degrees of disequilibrium may be detected between populations for clusters of closely linked genes (Zavattari et al. 2000). Moreover, differences in allelic compatibility may be most exaggerated after hybridization and backcrossing, leading to the rapid loss of unfavourable allelic complements. Disequilibrium is not expected to be as great among combinations of more favourably interacting alleles.

Recent selection history may also influence the rate of recombination within a strain. Increased levels of recombination have been reported in strains undergoing intense selection (Korol et al. 1994), and evolutionary theory predicts that mechanisms to increase recombination may be correlated responses to increased selection (Burt 2000). As a corollary to this, recently hybridized genomes may be expected to show increased recombination because they are effectively in a preselective state with respect to allelic combinations that may prove beneficial within future generations. In the current paper, we examine how hybridization may influence recombination level in a tetraploid derivative species of salmonid fish, the Arctic char (*Salvelinus alpinus*), and provide the first detailed genetic linkage map for the species.

The ancestor to extant salmonids experienced a doubling of all chromosomes resulting in tetraploidy (Dunn and Bennett 1967; Allendorf and Thorgaard 1984). Since this event (25–100 million years ago), the genome of salmonid fishes has been returning to a diploid state. This involves the divergence of the four homologous chromosomes into two distinct sets of homologous chromosomes (i.e., homeologous chromosomes). Diploidization is not complete in salmonid fishes and some chromosomes still pair with their ancestral counterparts by multivalent formation during meiosis in males. The separation of meiotic multivalents following recombination can result in "pseudolinkage" where an aberrant pattern of nonrandom assortment occurs and an excess of nonparental progeny types is observed when the phase of the alleles is known (Wright et al. 1983; Sakamoto et al. 2000). The observation of multivalent formations in males has been used to explain the lower recombination rates observed in this sex and the localization of recombination nodes (Allendorf and Thorgaard 1984; Sakamoto et al. 2000). Males show near-zero recombination for vast regions proximal to the centromere and strongly localized recombination in telomeric regions in some linkage groups (Sakamoto et al. 2000). The increased propensity for multivalent formations in hybrids (Davisson et al. 1973; Wright et al. 1983) suggests that the recombination rates in males may be affected by ancestry.

We compared linkage arrangements and recombination rates in two mapping panels of Arctic char derived from two genetically distinct races of Arctic char (Nauyuk and Fraser) in Canada. These crosses involved matings between a hybrid from these two strains and a pure strain parent. The use of backcross mapping panels in this study allows us to compare recombination differences between hybrid male and female genomes with levels observed in one of the pure strains. We also ascertained whether any of the mapping parents exhibited strong segregation distortion within linkage groups surveyed and if these regions were common to more than one parent. Markers showing segregation distortion may identify chromosomal regions of low interstrain genomic compatibility that influence hybrid fitness and survival (Danzmann and Gharbi 2001).

Materials and methods

Reference families

Two commercial strains of Arctic char were used to make the mapping panels. The Nauyuk strain was initiated with fish collected from Nauyuk Lake, Nunavut (formerly part of the Northwest Territories) in 1978 (de March 1993). The Fraser strain was initiated with fish collected from the Fraser River, Labrador, in 1980, 1981, and 1984 (de March 1993). The two strains are derived from fish belonging to two different phylogeographic groupings from the western (Nauyuk) and eastern Canadian Arctic (Fraser) (Brunner et al. 2001). There has been little genetic improvement for these aquaculture strains since their inception in 1978 and the early 1980s, and very few wild parents were used to initiate the original broodstocks (Nauyuk: three mating lots in 1978 involving 14 parents; Fraser: 1980 matings = 29 parents, 1981 matings = 40 parents, and 1984 matings = 4 parents). It is believed that most of the commercial strains were initiated from the 1984 matings, since these were the first crosses certified for commercial distribution. However, in

subsequent years, fish from the 1981 and 1980 matings were also distributed commercially (Lundrigan 2001). Two backcross families were used in map construction to maximize the amount of genetic variability. A Fraser strain male was crossed to a hybrid (Fraser × Nauyuk) female to create one family (family 2) and a hybrid (Fraser × Nauyuk) male was crossed to a Fraser strain female to produce the other mapping family (family 3). The genetic parental phase of both hybrids was unknown. Gametes were provided by Coldwater Fisheries (Coldwater, Ont.). In family 2, only 65 fish in total survived to 14 months postfertilization and 48 of these were sampled as mapping progeny. In family 3, 500 juvenile fish were subsampled from the progeny lot and successfully raised for 23 months postfertilization, and 48 of the largest fish from this family were sampled for the mapping family. The largest fish were chosen to minimize errors in sexing the fish through visual examination of their gonads. All animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Microsatellite and AFLP analysis

DNA was extracted from 50–100 mg of adipose fin, gill, or muscle using a phenol – chloroform – isoamyl alcohol protocol (Bardakci and Skibinski 1994). Microsatellite markers were PCR amplified according to the methods described in Woram et al. (2003). Amplified fragment length polymorphism (AFLP) analysis was performed as described by Vos et al. (1995). Digestion was performed using *Eco*RI and *Mse*I. Sixteen combinations of selective primers were used: two three-nucleotide extensions (AAG, ACG) were used for the *Eco*RI site and eight three-nucleotide extensions (CAA, CAC, CAG, CAT, CTA, CTC, CTG, CTT) were used for the *Mse*I site. AFLP fragments were amplified according to the methods described in Woram et al. (2003). PCR-amplified DNA was combined with 5 μL of loading buffer (formamide, bromophenol blue, EDTA), denatured at 95 °C for 10 min, and separated on a 6% polyacrylamide denaturing gel (7 M urea) using a model SA gel electrophoresis unit (GIBCO/BRL). Gels were run at 1500 or 1600 V for 1–3 h depending on fragment size. Gels containing fluorescent DNA were visualized by scanning with a fluorescent imaging system (i.e., Hitachi FMBIO). Allele sizes and AFLP bandsizes (in bp) were determined using FMBIO Analysis 8.0, which uses a logarithmic scale to compare amplified bands (alleles) with known basepair sizes: lane standard 350-TAMRA^{TM} (Applied Biosystems).

Genes

Genes were localized to linkage groups using single-nucleotide polymorphisms (SNPs) detected within the introns of genes with heteroduplex analysis (White et al. 1992) or by using microsatellite markers located within intron regions. PCR products were labeled by incorporating [^{32}P]dCTP during the reaction. Heteroduplexes were formed by heating the PCR product for 5 min at 95 °C and then cooling the product slowly to 20 °C over a period of 1 h. Products were separated on a 10% low cross-link (37.5:1) native polyacrylamide gel using 1× Tris–borate–EDTA buffer containing 10% glycerol. The loading buffer contained 50% glycerol, 5× Tris–borate–EDTA buffer, 0.2% bromophenol blue, and 0.2% xylene cyanol. Electrophoresis was conducted for 16 h at a constant power of 3 W, and heteroduplex products were visualized using autoradiography.

Trout red cell arrestin (*TRCArr*) and somatolactin (*SL*) were identified using polymorphisms at microsatellite loci. Metallothionein B (*MetB*), growth hormone 2 (*GH2*), β-actin, lysozymeA, lysozymeB, and peroxisomal proliferator-activated receptor (*PPAR-γ*) locations were determined by SNP analysis. *SOX*9 was localized using both SNPs and microsatellite markers. Two introns were detected in the *SOX*9 gene. Two microsatellite loci were detected within intron 1 (*SOX*9-msA, *SOX*9-msB) and SNP analysis detected three polymorphisms in intron 2 (*SOX*9-2a, *SOX*9-2b, *SOX*9-2c).

Genetic nomenclature

Naming of microsatellite markers follows the convention outlined by Jackson et al. (1998) and implemented by Sakamoto et al. (2000) and others. If microsatellite markers were designed and previously published, the naming convention according to the original publication was adopted (e.g., SSOSL32). Duplicated loci amplified by one primer set are identified by "/i" or "/ii" within the marker name (e.g., OmyFGT27/iTUF and OmyFGT27/iiTUF).

Naming of AFLP loci follows the convention where the three base selective primer extensions used to produce the loci are listed first followed by the basepair size of the locus. For example, AAG/CAA334 indicates that the three nucleotides (AAG) for the *Eco*RI primer and the three nucleotides (CAA) for the *Mse*I primer amplified a product at 334 bp. This is consistent with the nomenclature used by Young et al. (1998) and Spruell et al. (1999). Genes are identified with an italicized code referring to the gene name. The institution where the polymorphism was identified is listed in parentheses following the gene code.

Linkage analysis

Linkage analysis was performed using a compendium of programs (LINKMFEX, LINKGRP, MAPORD, GENOVECT, MAPDIS-V, and RECOMDIF) contained in LINKMFEX (version 1.6) (Danzmann 2000) (software may be obtained from the following web address: http://www.uoguelph.ca/~rdanzman/ software/). Initially, pairwise recombination distances among pairs of markers were calculated using the program LINKMFEX. Observed logarithm of the odds scores for each pair of markers were then used to cluster markers into their respective linkage groups at a logarithm of the odds threshold of either 3.0 or 4.0 using LINKGRP. Initial marker orders within linkage groups were ascertained using MAPORD and the chromosomal phase of each polymorphic marker set within a linkage group was obtained using GENOVECT. The phase-corrected raw genotype files were used to generate a virtual genetic map using MAPDIS-V. This program uses the observed phase scores of an individual and infers missing genotypes for marker orders within an individual using an algorithm that minimizes the overall recombination distances within a linkage group. This was accomplished two ways: first, by taking the initial estimate of marker order generated by MAPORD and, second, by using a ripple check in MAPDIS-V that retests each marker against all others in the linkage group. This identifies

potential marker relocalizations that are accepted assuming a minimization in overall map length within the linkage group tested. Map distances were assigned using recombination estimates between adjacent markers to account for the high level of interference observed in salmonid species (e.g., Allendorf et al. 1986). The map distance file generated was used to graphically generate a linkage map file using MAPCHART (Voorrips 2002) (software may be obtained from the following web address: http://www.joinmap.nl).

Many of the microsatellite markers tested were polymorphic in both mapping parents within a family and across both families. However, given the dominant/recessive nature of AFLPs, any given AFLP band was only informative in one of the two mapping parents within a family. Across families, a few AFLP markers were identified that had identical electrophoretic mobilities. These may reflect homologous chromosomal locations, although we recognize that such an interpretation is tentative until the actual DNA fragments are characterized by sequencing.

Segregation distortion

Segregation of alleles inherited from each parent at all polymorphic loci (excluding loci in which both parents were heterozygous for the same two alleles) was tested using a log likelihood adjusted χ^2 test to determine goodness of fit to the expected 1:1 segregation ratio using LINKMFEX. This test is appropriate for sample sizes between 25 and 200 (Sokal and Rohlf 1995). A Bonferroni correction was also applied to limit the experiment-wide error rate associated with multiple testing (Sokal and Rohlf 1995). Critical χ^2 values were calculated by dividing the alpha (0.05) by the number of linkage groups tested for each male and female mapping parent.

Recombination rates

Differences in recombination rate were estimated using 2×2 contingency G tests that compared parental versus recombinant genotypes inherited from each parent for a pair of linked markers, using the program RECOMDIF. Values of G were calculated for each pair of linked markers that were polymorphic in more than one parent. Williams' correction was used to correct for small sample size if the number of recombinants was less than five in any one cell (Sokal and Rohlf 1995). Bonferroni correction for multiple comparisons was applied as described above. An overall recombination ratio between a source and comparison parent was obtained as follows:

$$\frac{N_{sR}/N_s}{N_{cR}/N_c}$$

where N_s and N_c represent the total sample size (i.e., recombinants and nonrecombinants) among all pairwise markers compared within linkage groups between the source and comparison parent, respectively, and N_{sR} and N_{cR} represent the total number of recombinants detected among all pairwise comparisons for markers within known linkage groups in the source and comparison parent, respectively.

Results

Genetic linkage map

The linkage map for Arctic char currently has 327 genetic markers (184 microsatellite loci, 129 AFLPs, and 8 genes plus 1 gene cluster with 5 markers) and one phenotypic marker (SEX). Details of the map and the markers used in the study are available in an appendix (available at http://www.uoguelph.ca/~rdanzman/appendices/). A total of 40 linkage groups represented by 240 polymorphic markers (186 polymorphisms in the female and 123 in the male) were detected in family 2, and 37 linkage groups represented by 213 marker polymorphisms (109 in the female and 178 in the male) were detected in family 3. In total, 46 different linkage groups are described. However, 13 of these linkage groups are only represented by a pair of markers, and given the fact that 26 markers are still unlinked in the two mapping families, the current map linkage group designations for the smaller linkage groups must remain tentative.

Segregation distortion

Approximately 15.4% of the genetic markers examined (9.1% in family 2 and 21.7% in family 3) did not segregate according to 1:1 Mendelian expectations (Tables 1 and 2). However, assuming a more stringent Bonferroni threshold, these values were 1.6% and 4.2% of the markers in families 2 and 3, respectively. These markers were located on four linkage groups (7, 13, 20, and 43, with three markers unassigned). Interestingly, with a single exception, significant segregation distortion was only detected in the hybrid female (AC-13 and the three unassigned markers) and the hybrid male (AC-7 and AC-20). The female of family 3 also showed significant deviation for a single marker on linkage group 43.

Recombination differences

Map lengths showed considerable differences between the sexes. A comparison between the two most informative parents (i.e., the female of family 2 and the male of family 3) shows that the female map length is substantially greater (9.92 Morgans) compared with the male map length (3.90 Morgans). Average recombination rates differed significantly between the sexes in both mapping panels (see the appendix at http://www.uoguelph.ca/~rdanzman/appendices/). Average recombination rates between pairs of linked markers were 1.94 times greater (26 comparisons) and 1.43 times greater (50 comparisons) in female compared with male parents in families 2 and 3, respectively. Average recombination ratios should be substantially reduced when comparisons are made between markers that are linked in the female parent but unlinked in the comparison (i.e., male parent). Nine such instances were observed between the mapping parents in family 2 (female to male recombination ratio = 0.83) and six in family 3 (combined ratio = 0.89).

In all cases (except one) where significant differences (Bonferonni corrected) were detected in recombination rate between a pair of markers in the mapping parents, females had higher recombination levels. Markers in linkage groups 3, 20, 24, and 26 showed the greatest differences in recombination rate between the sexes in both families. In addition, higher female recombination levels were observed between

Table 1. Significant segregation distortion observed among the markers tested in mapping Arctic char family 2.

Linkage group[a]	Sex[b]	Marker	*N*	*G* test value	*P*[c]
4	F	Ots500NWFSC	48	4.14	<0.05
8	F	Omy26/iiINRA	48	5.44	<0.025
8	F	Str85INRA	48	6.92	<0.01
8	M	OMM1220/i	45	8.28	<0.005
13	F	OmyFGT27TUF	48	10.47	<0.005
13	F	Ssa85DU	48	14.87	<0.001*
20	F	OMM1268	46	4.33	<0.05
20	F	Sox9–2a(SFU)	48	10.47	<0.005
20	F	AAG/CTA368	48	5.44	<0.025
20	F	Sox9-msA(SFU)	48	10.47	<0.005
20	F	Sox9-msB(SFU)	48	4.14	<0.05
32	M	SSOSL32/ii	33	7.07	<0.01
UL-[8]	F	OMM1220/i	32	4.61	<0.05
UL	F	AAG/CAG432	48	23.29	<0.001*
UL	F	AAG/CTC227	48	23.29	<0.001*
UL	F	ACG/CAA135	48	17.42	<0.001*
UL	F	ACG/CTC65	48	4.14	<0.05
UL	F	ACG/CTC56	48	6.92	<0.01
UL-[32]	M	OMM1178	48	4.14	<0.05
UL	M	AAG/CTT304	47	6.29	<0.025

[a]A UL designation indicates that the marker is unlinked in the mapping parent indicated. Assignment to a linkage group (designated in square brackets) is based on recombination estimates from the additional mapping parents.
[b]F, female parent; M, male parent.
[c]An asterisk indicates significance following a Bonferroni correction.

markers on linkage groups 1, 7, 12, and 13 involving comparisons between a single pair of mapping parents (Tables 3 and 4).

In contrast with the large differences in recombination level detected between the sexes, within sex, very few significant differences were observed. Most of the these differences involved markers that were unlinked in the comparison parent and thus cannot unequivocally be considered indicative of a paired comparison because they may represent duplicated markers belonging to homeologous linkage groups. Only two marker combinations within linkage groups were comparable. On linkage group 7, the female from family 2 had higher recombination levels than those observed in the female from family 3. Alternatively, on linkage group 16, the male mapping parent from family 3 had higher recombination levels than the male parent in family 2. Although these comparisons were not significant at the more stringent Bonferonni-corrected level, they were indicative of the general differences in recombination level detected within sexes. Hybrid parents showed elevated levels compared with intrastrain parents. In the female comparison, the recombination ratio was 1.51 ($\chi^2 = 5.37$, $P < 0.025$) comparing female 2 with female 3 (see the appendix at http://www.uoguelph.ca/~rdanzman/appendices/). Female 2, the hybrid, had significantly elevated recombination levels. In the male comparison, the recombination ratio was 0.86 ($\chi^2 = 0.96$, $P > 0.05$) comparing male 2 with male 3 (see the appendix tables at http://www.uoguelph.ca/~rdanzman/appendices/). Although these differences were not significant, the hybrid male from family 3 had slightly elevated recombination levels. In addition, the within-family comparisons suggest that the hybrid female in family 2 had much higher recombination levels compared with the Fraser male parent (1.94:1 ratio) than the Fraser female had in comparison with the hybrid male in family 3 (1.43:1 ratio).

Homeologous linkage groups

We used the detection of duplicated microsatellite markers to tentatively identify ancestral homeologous affinities in the genome of Arctic char. Twenty-three homeologous linkage groups were identified (Table 5) based on the expression of 29 duplicated markers in the two mapping panels. Single homeologous affinities were evident for linkage groups 3, 7, 11, 12, 14, 15, 16, 23, 25, 28, 29, and 35, while two homeologous affinities were found with linkage groups 5, 13, 21, 24, 26, and 27. However, with linkage groups 7, 15, and 29, and one association with 26, the paired duplicate marker remains unassigned, and therefore, the homeologous affinity remains undetermined. For most linkage groups (i.e., 2, 9, 17–19, 22, and 30–46 (excluding 35)), no homeologies have been identified. In contrast, for some linkage groups (i.e., 1, 4, 6, and 8), more than two homeologous affinities were found.

Linkage group 20 appears to be a metacentric chromosome involving the fusion of two homeologous linkage groups. The evidence for this comes from a pair of duplicated markers (OMM1274/i with SalD100/iiSFU and OMM1274/ii with SalD100/iSFU) that map to opposite ends of the linkage group in the male of family 3 (see fig. 1 in the the appendix at http://www.uoguelph.ca/~rdanzman/appendices/). This suggests that these markers are found in the telomeric regions of a large metacentric chromosome. An al-

Table 2. Significant segregation distortion observed among the markers tested in mapping Arctic char family 3.

Linkage group[a]	Sex[b]	Marker	*N*	*G* test value	*P*[c]
1	F	OmyRGT35/iiTUF	48	4.14	<0.05
1	F	Sal16UoG	43	5.34	<0.025
4	F	AAG/CAC72	46	4.33	<0.05
6	M	One8ASC	48	4.14	<0.05
7	M	Omy301UoG	48	12.56	<0.001*
7	M	Omy325UoG	46	18.29	<0.001*
7	M	BHMS7.036/ii	47	6.29	<0.025
7	M	PPAR-γ(SFU)	42	9.92	<0.005
9	M	AAG/CAC271	45	5.10	<0.025
9	M	Ssa14DU	48	4.14	<0.05
13	F	OMM1194	47	7.91	<0.005
13	F	OMM1211	48	6.92	<0.025
14	F	Sco19UBC	48	5.44	<0.025
14	F	AAG/CTG97	46	5.68	<0.025
14	M	One11ASC	48	6.92	<0.01
14	M	Sco19UBC	48	6.92	<0.01
14	M	AAG/CTT185	48	5.44	<0.025
14	M	SalP61SFU	47	7.91	<0.005
20	M	OMM1268	46	15.60	<0.001*
20	M	OMM1274/i	48	10.47	<0.001*
20	M	OmyRGT30TUF	48	12.56	<0.001*
20	M	OmyRGT4TUF	48	12.56	<0.001*
20	M	GH2(SFU)	41	7.27	<0.01
20	M	Sox9–2a(SFU)	44	11.51	<0.001*
20	M	AAG/CTG316	44	5.95	<0.025
20	M	Sox9–2b(SFU)	40	6.58	<0.025
20	M	Sox9-msA(SFU)	45	12.33	<0.001*
20	M	Sox9-msB(SFU)	45	6.58	<0.025
22	F	SL/i(INRA)	46	5.44	<0.025
22	F	AAG/CAC99	48	5.68	<0.025
24	M	OmyRGT11TUF	48	4.14	<0.05
24	M	OmyRT7/iiTUF	48	4.14	<0.05
24	M	SalF41/iiSFU	46	4.33	<0.05
24	M	One1ASC	48	4.14	<0.05
26	F	OMM1205/i	47	4.87	<0.05
26	F	Cocl3LAV	48	5.44	<0.025
26	F	One10ASC	48	8.59	<0.005
26	F	SalD25SFU	46	5.68	<0.025
26	F	Ots531NWFSC	40	5.00	<0.05
26	M	OMM1231/ii	47	4.87	<0.05
43	F	OMM1270	48	12.56	<0.001*
UL-[26]	F	OMM1231/i	47	4.87	<0.05
UL	F	Sfo23LAV	48	8.59	<0.005
UL	F	AAG/CAA176	48	4.14	<0.05
UL	F	ACG/CTT310	46	4.33	<0.05
UL	M	Ssa6DIAS	45	8.28	<0.005
UL-[44]	M	β-actin(SFU)	45	8.28	<0.005

[a]A UL designation indicates that the marker is unlinked in the mapping parent indicated. Assignment to a linkage group (designated in square brackets) is based on recombination estimates from the additional mapping parents.
[b]F, female parent; M, male parent.
[c]An asterisk indicates significance following a Bonferroni correction.

ternative possibility, that this linkage group represents pseudolinked segments, cannot be rejected at present, as the linkage data are derived from a male mapping parent. However, duplicated pseudolinked markers have previously been observed to map adjacent to one another in rainbow trout (Sakamoto et al. 2000) and not to opposite ends of the chromosome. Genetic mapping of these two duplicated markers to opposite ends of the same female linkage group would de-

Table 3. Significant differences in female compared with male recombination rates between common marker pairs within linkage groups of Arctic char using progeny derived within families.

Linkage group[a]	Female	Male	Locus 1	Locus 2	Ratio F:M[b]	*N*	*G* test value	*P*[c]
7	Fam2	Fam2	Omy325UoG	Ots3BML	9.09	90	7.75	<0.01
7	Fam2	Fam2	Omy301UoG	Ots3BML	Undefined	90	8.04	<0.005*
11	Fam2	Fam2	OmyRGT35/iTUF	SalF56SFU	Undefined	88	6.56	<0.01
12, UL	Fam2	Fam2	OMM1236	Sal9UoG	0.52	94	5.02	<0.025
13	Fam2	Fam2	BHMS437	OMM1211	Undefined	27	4.07	<0.05
13, UL	Fam2	Fam2	OMM1194	OMM1211	0.13	63	9.44	<0.005*
14	Fam2	Fam2	Sco19UBC	One11ASC	Undefined	96	6.53	<0.025
14	Fam2	Fam2	Sco19UBC	BHMS238	Undefined	92	5.07	<0.025
23, UL	Fam2	Fam2	Ots516/iNWFSC	BHMS373	0.14	92	19.45	<0.001*
23, UL	Fam2	Fam2	Ots516/iNWFSC	OmyRT16TUF	0.31	54	6.94	<0.01
23, UL	Fam2	Fam2	BHMS373	BHMS272/ii	0.43	70	4.57	<0.05
24	Fam2	Fam2	SalF41/iiSFU	OmyRT7/iiTUF	19.65	84	24.06	<0.001*
26	Fam2	Fam2	OMM1231/i	Cocl3LAV	0.18	95	7.32	<0.01
26	Fam2	Fam2	SsaF43NUIG	Cocl3LAV	0.20	93	6.13	<0.025
32, UL	Fam2	Fam2	OMM1178	OMM1276	0.39	88	6.94	<0.01
1	Fam3	Fam3	OMM1300	Omy21INRA	Undefined	86	12.76	<0.001*
1, UL	Fam3	Fam3	Ots523/iNWFSC	Sal16UoG	0.14	56	13.18	<0.001*
3	Fam3	Fam3	SalF41/iSFU	Ots1BML	Undefined	80	9.64	<0.005*
3	Fam3	Fam3	BHMS490	BHMS431	Undefined	46	11.86	<0.001*
13, UL	Fam3	Fam3	OMM1194	OMM1211	0	94	24.54	<0.001*
13	Fam3	Fam3	Ssa85DU	OmyPuPuPyDU	Undefined	94	4.92	<0.05
20	Fam3	Fam3	OMM1274/i	SalD100/iSFU	0	84	7.99	<0.005
20	Fam3	Fam3	OMM1274/ii	SalD100/iiSFU	0.12	63	9.02	<0.005
20	Fam3	Fam3	OMM1274/ii	OMM1268	3.84	92	15.27	<0.001*
20	Fam3	Fam3	OMM1274/ii	OmyRGT4TUF	3.83	96	14.99	<0.001*
23, UL	Fam3	Fam3	BHMS276	Ots516/iNWFSC	0	94	36.11	<0.001*
23, UL	Fam3	Fam3	Ots516/iNWFSC	OmyRT16TUF	0.30	52	4.99	<0.05
23	Fam3	Fam3	BHMS276	Omy7INRA	10.0	40	10.66	<0.005*
26	Fam3	Fam3	SalD25SFU	OMM1231/ii	7.04	90	5.17	<0.025
26	Fam3	Fam3	SalD25SFU	Ots531NWFSC	4.0	80	4.14	<0.05
26	Fam3	Fam3	One10ASC	Cocl3LAV	0.21	96	5.14	<0.025
26	Fam3	Fam3	OMM1231/ii	Cocl3LAV	0	94	15.91	<0.001*

[a]A UL designation indicates that the pair of markers indicated are unlinked in the male mapping parent.
[b]Undefined indicates that the recombination level in the male mapping parent is zero. The recombination ratio in such instances is taken as the recombination distance in the first parent.
[c]An asterisk indicates significance following a Bonferroni correction.

finitively establish this as a metacentric chromosome composed of fused homeologues.

Discussion

We report the first detailed linkage map for Arctic char, which possesses predominantly acrocentric type chromosomes. As such, it will give a reference framework in which to compare chromosomal rearrangements within the family Salmonidae. Map distances span approximately 10 and 4 Morgans in our most completely genotyped female (186 markers) and male (178 markers) mapping parents, respectively (262 markers total). Comparable results using outcrossed mapping parents have been obtained in other salmonid species based on genotyping efforts with similar numbers of markers. For example, female map distances were reported to span 10 Morgans compared with 4.6 Morgans for the male linkage map (209 markers) in rainbow trout (Sakamoto et al. 2000). In brown trout, Gharbi (2001) reported a female map distance of 9.2 Morgans compared with 3.7 Morgans for the male linkage map (247 markers). Young et al. (1998) reported a total male generated map length of approximately 20 Morgans (476 markers) using a mapping panel of doubled haploid androgenetic individuals derived from a hybrid between clonal lines of rainbow trout. Recently, this map has been updated to include 1360 markers spanning 46 Morgans (Nichols et al. 2003).

Homeologous linkage groups

Previous studies have identified homeologous linkage groups in rainbow trout (Sakamoto et al. 2000; O'Malley et al. 2003; Nichols et al. 2003) and brown trout (Gharbi 2001) based on the joint segregation of several duplicated microsatellite markers. Because of their tetraploid ancestry, salmonid linkage groups share homeologous affinities as well as homologous affinities to one another. Each pair of homologous chromosomes is expected to possess complementary syntenic regions on other linkage groups within the genome of each salmonid species dependent on the

Table 4. Significant differences in female compared with male recombination rates between common marker pairs within linkage groups of Arctic char comparing progeny between mapping families.

Linkage group[a]	Female	Male	Locus 1	Locus 2	Ratio F:M[b]	*N*	*G* test value	*P*[c]
1	Fam2	Fam3	Omy27/iDU	Ssa77NUIG	6.25	96	3.95	<0.05
1, UL	Fam2	Fam3	OMM1201	Ots523/iNWFSC	0.37	63	5.14	<0.025
3	Fam2	Fam3	Ots101SSBI	SalF41/iSFU	Undefined	89	12.52	<0.001*
4	Fam2	Fam3	OmyRT8/iTUF	ACG/CTA162	6.59	93	4.76	<0.05
4	Fam2	Fam3	Omy6DIAS	ACG/CTA162	Undefined	93	7.66	<0.01
5	Fam2	Fam3	Ots517NWFSC	Ots516/iNWFSC	0.33	70	4.54	<0.05
7	Fam2	Fam3	Omy325UoG	Ots3BML	Undefined	67	7.40	<0.01
7	Fam2	Fam3	BHMS7.036/ii	Omy325UoG	0	91	5.16	<0.025
7	Fam2	Fam3	Omy301UoG	Ots3BML	Undefined	68	4.82	<0.05
11	Fam2	Fam3	OmyRGT35/iTUF	Omy16DIAS	Undefined	96	5.06	<0.025
12	Fam2	Fam3	OMM1236	Omy77DU	Undefined	90	10.53	<0.005*
13	Fam2	Fam3	BHMS437	OMM1211	Undefined	25	4.08	<0.05
13	Fam2	Fam3	OmyFGT27TUF	OMM1211	Undefined	38	11.28	<0.001*
13	Fam2	Fam3	Ssa85DU	OMM1211	Undefined	68	22.86	<0.001*
13, UL	Fam2	Fam3	OMM1194	OMM1211	0.16	65	6.48	<0.025
14	Fam2	Fam3	Sco19UBC	One11ASC	Undefined	96	6.53	<0.025
20	Fam2	Fam3	SOX9–2a(SFU)	OmyRGT4TUF	5.67	91	8.14	<0.005
20	Fam2	Fam3	SOX9–2a(SFU)	OmyRGT30TUF	6.27	90	9.69	<0.005
20	Fam2	Fam3	SOX9-msA(SFU)	OMM1268	6.61	89	4.76	<0.05
20	Fam2	Fam3	SOX9-msA(SFU)	OmyRGT4TUF	11.59	92	11.46	<0.001*
20	Fam2	Fam3	SOX9-msA(SFU)	OmyRGT30TUF	18.16	91	13.23	<0.001*
20	Fam2	Fam3	GH2(SFU)	AAG/CTG316	Undefined	86	9.37	<0.005
20	Fam2	Fam3	OMM1268	OmyRGT4TUF	Undefined	91	5.16	<0.025
20	Fam2	Fam3	OMM1268	OmyRGT30TUF	Undefined	90	6.76	<0.01
23, UL	Fam2	Fam3	BHMS276	Ots516/iNWFSC	0	95	36.61	<0.001*
24	Fam2	Fam3	SalF41/iiSFU	Ots510NWFSC	6.47	90	4.19	<0.05
24	Fam2	Fam3	SalF41/iiSFU	OmyRT7/iiTUF	Undefined	88	32.69	<0.001*
24	Fam2	Fam3	Ots510NWFSC	OmyRT7/iiTUF	15.55	93	16.02	<0.001*
26	Fam2	Fam3	SalD25SFU	OMM1231/i	7.04	90	5.16	<0.025
26	Fam2	Fam3	SalD25SFU	SsaF43NUIG	Undefined	91	9.72	<0.005
26	Fam2	Fam3	OMM1231/i	Cocl3LAV	0.18	94	7.54	<0.01
26	Fam2	Fam3	SsaF43NUIG	Cocl3LAV	7.74	94	5.94	<0.025
31	Fam2	Fam3	OMM1290	ACG/CTG204	Undefined	93	9.70	<0.005
32, UL	Fam2	Fam3	OMM1178	OMM1276	0.35	90	9.61	<0.005
33, UL	Fam2	Fam3	SsaR15LEE	AAG/CAA84	0.36	94	8.29	<0.005
1, UL	Fam3	Fam2	OmyFGT27/iiTUF	Omy21INRA	0.24	83	13.56	<0.001*
7	Fam3	Fam2	Omy301UoG	Ots3BML	Undefined	93	6.24	<0.025
13, UL	Fam3	Fam2	OMM1184	OMM1211	0	92	25.27	<0.001*
13, UL	Fam3	Fam2	OMM1194	OMM1211	0	92	31.29	<0.001*
23, UL	Fam3	Fam2	Ots516/iNWFSC	OmyRT16TUF	0.24	53	8.56	<0.005
26, UL	Fam3	Fam2	One10ASC	OMM1205/i	0.09	93	25.22	<0.001*
26, UL	Fam3	Fam2	One10ASC	Cocl3LAV	0.09	95	23.22	<0.001*
26, UL	Fam3	Fam2	One10ASC	Ots531NWFSC	0.21	87	14.76	<0.001*
26, UL	Fam3	Fam2	OMM1205/i	Cocl3LAV	0	94	22.75	<0.001*
26, UL	Fam3	Fam2	OMM1205/i	Ots531NWFSC	0.18	86	8.09	<0.005
26, UL	Fam3	Fam2	One10ASC	OMM1231/i	0.10	94	19.30	<0.001*
26, UL	Fam3	Fam2	OMM1205/i	OMM1231/i	0	94	24.54	<0.001*
26	Fam2	Fam2	OMM1231/i	Cocl3LAV	0	95	15.65	<0.001*

[a]A UL designation indicates that the pair of markers indicated are unlinked in the male mapping parent.
[b]Undefined indicates that the recombination level in the male mapping parent is zero. The recombination ratio in such instances is taken as the recombination distance in the first parent.
[c]An asterisk indicates significance following a Bonferroni correction.

polyploid origin of these sets. It has been suggested that the degree to which these homeologous regions have diverged is largely dependent on the degree of crossing over experienced by the extant homeologues during meiosis (Wright et al. 1983). Regions that experience the greatest degree of homeologue exchange (i.e., telomeric segments of chromo-

Table 5. Potential homeologous linkage group affinities detected in Arctic char using the duplicated DNA markers shown.

Putative homeologues[a]	Duplicated markers
AC-1 and AC-11	OmyRGT35TUF
AC-1 and AC-13	OmyFGT27TUF
AC-1 and AC-21	Omy1UoG, Omy27DU
AC-1 and AC-35	Ots523NWFSC
AC-3 and AC-24	BHMS161, OmyRT7TUF, SalF41SFU
AC-4 and AC-28	OmyRT8TUF
AC-4 and AC-25	SSOSL32
AC-5 and AC-23	Ots516NWFSC
AC-6 and AC-8	BHMS206, Omy26INRA
AC-6 and AC-12	Ots501NWFSC
AC-6 and AC-10/23[b]	BHMS272
AC-6 and AC-26	OMM1205
AC-7 and UL	BHMS7.036
AC-8 and AC-21	Ots2BML
AC-8 and AC-24	OMM1220
AC-12 and AC-27	Str60INRA
AC-13 and AC-14	Ssa3DIAS
AC-15 and UL	OmyRGT2TUF
AC-16 and AC-27	BHMS417
AC-20 and AC-20	OMM1274, SalD100SFU, *SOX9–2*, *SOX9-ms*
AC-26 and UL	OMM1231
AC-29 and UL	OMM1167

[a]UL indicates that one of the duplicated copies for the marker shown is currently unlinked to any described linkage group.

[b]The assignment for BHMS272 is currently unresolved, as duplicated copies map to AC-10 and AC-23 in the family 3 mapping male. A single marker maps to AC-6 in the family 3 mapping female.

somes) are expected to retain the highest level of homeologue affinity (Allendorf and Thorgaard 1984).

Given the fundamental number of chromosome arms in salmonid fishes (i.e., 100–104) and their tetraploid ancestry, it is predicted that 25–26 homeologous sets of chromosomes should exist within the genome of each species if homeologous arms are preserved during the diploidization process. In the current study, it would appear that close to this number of homeologous sets have been identified; however, it also appears that some linkage groups are represented by more than two homeologous affinities and that many linkage groups have not been identified as belonging to a homeologous grouping. If there have only been whole-arm translocations without subsequent rearrangements within each linkage group arm, then any one linkage group should have no more than two homeologous pairings (i.e., assuming the linkage group is a metacentric chromosome formed from two whole-arm fusions). Since the genome of Arctic char is primarily composed of acrocentric chromosomes (Hartley 1991), most homeologous pairings should be represented as single homeologies. The observation that some linkage groups may possess homeologies with more that two other linkage groups could indicate that partial arm translocations occurred in the evolutionary history of this species, although this cannot be firmly established with the present low-resolution map.

Gene order conservation

The identification of at least two *SOX*9 genes in Arctic char is consistent with the discovery of two *SOX*9 genes in zebrafish (Chiang et al. 2001). However, the detection of two copies of the gene in zebrafish suggests that four copies should be present in salmonids because of their tetraploid origin subsequent to the postulated genome duplication of all teleosts (Aparicio 2000). It is possible that four loci were initially present and one pair diverged during diploidization to an extent that they are no longer detectable with primers designed for the alternate pair of loci. Alternatively, the additional loci may have been lost or silenced within the genome of Arctic char. In zebrafish, linkage analysis determined that the two *SOX*9 genes map to different linkage groups (linkage groups 3 and 12) that share at least six duplicated copies of genes (Chiang et al. 2001). Unfortunately, the other two type I genes located on AC-20 in Arctic char (*TRCArr* and *GH2*) have not been mapped in zebrafish, so it is not possible to compare orthologous relationships. However, all three type I genes that we detected on AC-20 are conserved on a single linkage group (i.e., linkage group 11) in the mouse (*Mus musculus*) (*SOX*9 = segment 11E2, *Gh* = segment 11E1, LOC216869 (putative *Arrestin-Beta2*) = segment 11B3) (NCBI UniGENE search), suggesting conserved orthologous syntenies. The arrestin gene assignment is tentative, however, given the multiple arrestin gene forms that have been identified in vertebrates.

Segregation distortion

When markers were identified in Arctic char with both significant ($P < 0.001$) and marginal ($P < 0.005$) segregation distortion, it was evident that in most cases (20 of 25), the hybrid parent (female in family 2 and male in family 3) exhibited the distortion. Such differences suggest that semilethal effects may be more evident in hybrid versus intrastrain genomes of salmonids. The functional basis for increased rates of segregation distortion in hybrids is not clearly established, although previous studies in salmonids suggest that epistatic interactions among alleles derived from different genomic backgrounds may influence the survival and fitness of hybrids. For example, incompatibilities in the performance expression of upper thermal tolerance quantitative trait locus alleles in backcross hybrids of rainbow trout have been reported (Danzmann et al. 1999). Survival performance was elevated in backcross hybrids receiving intrastrain alleles from the F_1 hybrid parent in the cross compared with backcross progeny receiving interstrain alleles. Similarly, in an F_2 population between two cultivars of rice, Fukuta et al. (2000) reported that of the 12 linkage groups showing significant segregation distortion, 10 were represented by an excess of intrastrain homozygous genotypes, while only two possessed an excess of interstrain heterozygotes. Similar effects may be occurring in the current mapping families. Unfortunately, since the allelic phases were unknown in our hybrid parents, we could not directly test for such epistatic interactions.

Since a biased subsample of fish was used for analysis in family 3 (i.e., the largest surviving fish), it was postulated that differences in segregation distortion detected within this family could possibly result from the differential association of quantitative trait locus alleles for growth in the selected

individuals. We observed that the majority of markers demonstrating significant distortion in family 3 are located on AC-20. This linkage group houses *GH2*, and since it appears to be a metacentric composed of two fused homeologues, it presumably also possesses the *GH1* locus (which was not genotyped in this study). Thus, these genes are obvious candidates for associations with growth. Also, given the paucity of allelic variation that occurs in both the Nauyak and Fraser strains (Lundrigan 2001), we considered that homozygous progeny (or progeny possessing only alleles detected in the Fraser strain) would be indicative of intrastrain genomic combinations. When comparisons were made on this basis, it was observed that at four of five loci examined on AC-20, apparent intrastrain allelic combinations significantly exceeded interstrain allelic combinations. The occurrence of apparent higher intrastrain allelic expression was also detected at one other linkage group (AC-7). Although the interpretations that we have applied to the existing data must remain tentative, since allelic phases were not unequivocally established, the findings do support the general interpretation that segregation distortion may be more elevated in hybrid genomes within regions experiencing selection. In our study, artificial selection on fish body size has resulted in the apparent increased segregation distortion of marker alleles within a linkage group that presumably has a strong influence on growth.

Recombination rate differences

The fact that the largest proportion of the significant differences in recombination rate between the sexes were detected between linked markers in the female parent but unlinked markers in the male parent (13 of 21 comparisons) suggests that these markers may (*i*) be telomerically located on the male linkage maps, (*ii*) represent cross-amplified duplicated markers that may actually assign to homeologous linkage groups in the future, (*iii*) represent regions of localized chiasmata formation in the male linkage group (i.e., markers in these regions may appear unlinked in male maps; see Sakamoto et al. 2000), or (*iv*) represent regions of chromosomal arm polymorphisms within the species.

In all cases except one (OMM1231/ii with Cocl3LAV on AC-26) in which the pair of markers being compared between the sexes mapped syntenically on the same linkage groups, females had significantly higher recombination rates than males. The exception on AC-26 may in fact represent a comparison within the telomeric region of AC-26, reflecting higher male recombination levels in these regions. Cocl3LAV maps telomerically in the genome of rainbow trout (unpublished data). If this arrangement is conserved in Arctic char, a telomeric location could account for observed sex-specific differences in the present study.

Sakamoto et al. (2000) and Gharbi (2001) found that female recombination rates were higher than those of males around the centromere whereas male recombination rates were higher than those of females in telomeric regions. Decreased recombination often results in a clustering of markers to one location on the linkage map. Young et al. (1998) and Nichols et al. (2003) inferred the location of centromeres in an androgenetically derived family of rainbow trout based on the previous observation of clustering of AFLP markers around the centromere of corn and soybean. One distinct cluster was observed on each linkage group. A similar inference may be made with the Arctic char linkage map. Thus far, each male derived linkage group (with the exception of AC-20) contains only one predominant cluster of genetic markers. If such clusters accurately predict centromeric positions in linkage groups, significant differences in recombination rate between male and female Arctic char follow patterns similar to those observed in other salmonid species that have been genetically mapped (i.e., brown trout (Gharbi 2001) and rainbow trout (Sakamoto et al. 2000)).

Sakamoto et al. (2000) postulated that the hybrid nature of the male mapping parents used to construct a linkage map of rainbow trout enhanced the large sex-specific differences in recombination rate that were observed. They suggested that the two F_1 hybrid males that they used in their mapping panels may have experienced a reduction in recombination rate because of genetic incompatibilities among homologous chromosome segments, which may have prevented crossing over. This would in turn inflate recombination rate differences with the intrastrain females that were used. In the present study, we directly tested sex-specific differences in recombination level between hybrid and pure strain parents. Recombination levels were elevated in the hybrid parents and this difference was significant in the female comparison. The lack of a similar result for males may relate to the fact that recombination levels are greatly reduced in males throughout most chromosomal regions excluding the telomeres. Thus, a comparison of recombination levels in males needs to focus on specific differences in telomeric recombination rates.

These findings do not support the proposition that hybridization may reduce recombination rates within the hybrid individual. Outcrossing in salmonids may in fact elevate general recombination levels in the hybridized genomes of the new strain, at least for the first few generations following hybridization. The generality of this statement awaits further empirical testing in other species and across more strains. In addition, a direct comparison of recombination rate differences among salmonid species awaits the direct completion of more detailed genetic maps and empirical evaluation of recombination differences in pairwise comparisons between linked homologous chromosome segments.

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